BOX PCT

ATTORNEY'S DOCKET NO: 24669

U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE		DATE: 07 June 2001 (07.06.2001)
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLN. NO. (if known): Not Yet Assigned 09/857554
INTERNATIONAL APPLICATION NO.: PCT/EP99/09633	INTERNATIONAL FILING DATE: 08 December 1999 (08.12.99)	PRIORITY DATE CLAIMED: 09 December 1998 (09.12.98)

TITLE OF INVENTION: **ACTIVE INGREDIENT MATRIX IN THE FORM OF A BIOLOGICALLY RESORBABLE, POROUS NONWOVEN, METHOD FOR ITS MANUFACTURE AND USE THEREOF**

APPLICANT(S) FOR DO/EO/US: SCHOLL, Edmund

Applicant hereby submits to the Unites States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. __ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. X This express request to begin national examination procedures (35 USC 371(f) The submission must include items(5), (6), (9) and (21) indicated below.

4. X A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2)):

   a. X is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ has been communicated by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. X A English translation of the International Application as filed (35 U.S.C. 371(c)(2).

7. X Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

   a. __ are attached hereto (required only if not transmitted by the International Bureau).
   b. __ have been communicated by the International Bureau.
   c. __ have not been made; however, the time limit for making such amendments has NOT expired.
   d. X have not been made and will not be made.

8. __ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ___ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. X A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11 to 20 BELOW CONCERN OTHER DOCUMENT(S) OR INFORMATION INCLUDED:

11. __ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ___ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. X A **FIRST** preliminary amendment.
14. ___ A **SECOND** or **SUBSEQUENT** preliminary amendment
15. ___ A substitute specification.
16. ___ A change of power of attorney and/or address letter.
17. ___ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter2 and 35 USC 1821 - 1825
18. ___ A second copy of the published international application under 35 USC 154(d)(4)
19. ___ A second copy of the English language translation of the international application under 35 USC 154(d)(4)
20. X Other items or information:

TRANSMITTAL FORM; FEE CALCULATION; INTERNATIONAL PUBLICATION WO 00/33822; INTERNATIONAL PUBLICATION DATE 15 JUNE 2000 WITH VERIFIED ENGLISH TRANSLATION CONSISTING OF 28 PAGES INCLUDING 14 PAGES TEXTUAL SPECIFICATION, 3 PAGES OF 22 CLAIMS; 1 SHEET CONTAINING THE ABSTRACT; 0 SHEETS DRAWINGS; PCT/ISA/210 INTERNATIONAL SEARCH REPORT; TRANSLATION OF AMENDED SHEETS TO INTERNATIONAL PRELIMINARY EXAMINATION REPORT; PRELIMINARY AMENDMENT TO AMENDED SHEETS OF IPER **TO B E EXAMINED** WITH CLEAN COPY; UNEXECUTED INVENTOR'S DECLARATION; PCT/RO/101 REQUEST PCT/IB/304 NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENT; PCT/IB/332 INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION; PCT/IPEA/402 NOTIFICATION OF RECEIPT OF DEMAND.

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U.S. APPLICATION NO. (if known) not yet assigned	INTERNATIONAL APPLICATION NO.	DATE: 07 June 2001 (07.06.2001)
09/857554	PCT/EP99/09633	

17. x The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO:.....$860.00	$860.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482).....$690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1000.00		
International preliminary examination fee (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NO. FILED	NO. EXTRA	RATE		
TOTAL	34 -20=	14	X $ 18.00	$	**252.00**
INDEPENDENT	2 - 3=	02	X $ 80.00	$	0.00
Multiple dependent claims(s) (if applicable)			+ $260.00	$	0.00
			TOTAL OF ABOVE CALCULATIONS =	$	**1,112.00**
Reduction by ½ for asserting small entity, if applicable. (Note 37 CFR 1.9, 1.27, 1.28).				$	**0.00**
			SUBTOTAL =	$	1,112.00
Processing fee of **$130.00** for furnishing the English translation later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	0.00
			TOTAL NATIONAL FEE =	$	1,112.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property +**				$	**0.00**
			TOTAL FEES ENCLOSED =	$	**1,112.00**
			Amount to be: refunded		$
			charged		$

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a. X One check in the amount of $1,112.00 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. 14-0112 in the amount of $_____ to cover the above fees. (A duplicate copy of this sheet is enclosed.)

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0112.

d. Fees are to be charged to a credit card ____ WARNING: Information on this form may become public ____Credit Card Information should not be included on this form. ___ Provide credit card information and authorization on PTO-2038 ___

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed to request that the application be restored to pending status.

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Rev. 02/98

Attorney Docket No. 24669

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
SCHOLL, Edmund
International Application No. PCT/EP99/09633
Serial No. NOT YET ASSIGNED
International Filing Date: 8 December 1999 (08.12.99)
Filed: June 7, 2001
For: **ACTIVE INGREDIENT MATRIX IN THE FORM OF A BIOLOGICALLY RESORBABLE, POROUS NONWOVEN, METHOD FOR ITS MANUFACTURE AND USE THEREOF**

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examining on the merits and calculating the filing fee for the national phase application filed herewith, please enter the following amendments:

IN THE CLAIMS:

Please cancel the claims 1-21 from the translated annexed sheets to the International Preliminary Examination Report of the captioned application and enter the newly submitted set of claims as attached (Attachment A) with this preliminary amendment.

REMARKS

The above amendments have been made to remove multiple dependencies from the claims and to conform them to U.S. practice. No new matter has been added. Pursuant to the new rules implementing the AIPA, a clean copy of the new claims is attached per Attachment A.

Respectfully submitted,

NATH & ASSOCIATES PLLC

By: _____
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ATTACHMENT A - NEW CLAIMS

CLAIMS

--22. An active ingredient matrix: in the form of a biologically resorbable, porous nonwoven of collagen fibrils in lyophilized form with a retarded release of active ingredients, containing at least one homogeneously distributed active ingredient poorly soluble in water and body fluids, which, apart from the collagen fibrils as the carrier structure and the at least one active ingredient, is substantially free from further constituents, which is substantially free from salt and in which the at least one active ingredient in physiological medium has a solubility of< 10 mg/ml.

23. The active ingredient matrix according to claim 22, wherein it has a layer thickness of 0.5 to 15 mm.

24. The active ingredient matrix according to claim 22, wherein it has a density of 12 to 180 mg/cm3.

25. The active ingredient matrix according to claim 22, wherein it has a pore volume of 60 to 80% of the total volume.

26. The active ingredient matrix according to claim 22 wherein it has an average pore size in the range of 20 to 150 µm.

27. The active ingredient matrix according to claim 22 wherein it has an air permeability of 2500 to 5000 ml/cm2/min for a layer thickness of 4.2 mm.

28. The active ingredient matrix according to claim 22, wherein the at least one poorly soluble active ingredient is a medicament.

29. The active ingredient matrix according to claim 28, wherein the medicament is an antibiotic.

30. The active ingredient matrix according to claim 22 wherein in addition to the at least one poorly soluble active ingredient, it contains at least one less poorly soluble or easily soluble active ingredient.

31. A method for the manufacture of biodegradable active ingredient matrix in the form of an open-cell nonwoven or sponge of uncrosslinked, resorbable collagen fibrils, for the manufacture of an active ingredient matrix according to claim 22, wherein pieces of cleaned, degreased and dried hide are allowed to swell in dilute, aqueous solutions of organic acids until an elastic material is

obtained, the swollen pieces are rinsed several times with aqueous media, until the pH-value is increased, the rinsed pieces are mechanically separated into fibers for forming a suspension of collagen fibrils, the pourable collagen suspension having a pH-value of > 3.5 to < 4.8 is mixed with at least one difficultly soluble active ingredient in finely divided form and homogenized and the active ingredient-containing suspension is then lyophilized to the nonwoven or sponge.

32. The method according to claim 31, wherein the concentration of the organic acid used for swelling and the number of rinsing operations are chosen and matched to one another in such a way that following the rinsing and separation into fibers, without prior pH-correction, a collagen suspension is obtained with a pH-value of> 3.5 to <4.8.

33. The method according to claim 31, wherein the rinsing operation covers at least two rinsing cycles.

34. The method according to claim 32, wherein rinsing is performed for 5 to 60 hours.

35. The method according to claim 32, wherein for swelling purposes use is made of an acid solution with an acid concentration of 0.01 to 2 N.

36. The method according to claim 32, wherein the hide portions are swollen in the organic acid to 3 to 10 times their weight.

37. The method according to claim 32, wherein after rinsing and removing the rinsing water, the swollen collagen granulate is transformed by the addition of water into a 0.1 to 10 % mixture, based on the dry collagen material weight and this mixture is homogenized by dispersion to the collagen suspension, the fiber union of the collagen fibrils being broken.

38. The method according to claim 32, wherein the at least one poorly soluble active ingredient is added in finely divided form.

39. The method according to claim 32, wherein the suspension of the collagen fibrils, following the addition of the at least one difficultly soluble active ingredient, is homogenized for uniform distribution of the at least one active ingredient in the suspension.

40. The method according to claim 32, wherein apart from the at least one difficultly soluble active ingredient, at least one less poorly soluble active ingredient is added.

41. The method according to claim 32, wherein the homogenized, active ingredient-containing collagen suspension is lyophilized without any further intermediate treatment to areal nonwovens.

42. Use of the active ingredient matrix according to claim 22 as an implantable and completely resorbable depot for active ingredients with a retarded active ingredient delivery.

43. The active ingredient matrix according to claim 23, wherein the layer thickness is 2 to 5 mm.

44. The active ingredient matrix according to claim 27, wherein the air permeability is 2700 to 3400 ml/cm2/min.

45. The active ingredient matrix according to claim 28, wherein the antibiotic is one or more of aminoglycoside antibiotics.

46. The active ingredient matrix according to claim 45, wherein the aminoglycoside antibiotics are selected from the group of clindamicin-palmitate, clindamicin-palmitate hydrochloride and gentamicin-crobefat.

47. The method according to claim 31, wherein the aqueous medium is demineralized water.

48. The method according to claim 32, wherein the pH-value is 4 to 4.5.

49. The method according to claim 33, wherein the rinsing operation covers at least five rinsing cycles.

50. The method according to claim 34, wherein rinsing is performed for 6 to 48 hours.

51. The method according to claim 35, wherein the acid concentration is 0.05 to 0.5 N.

52. The method according to claim 36, wherein the hide portions are swollen to 4 to 8 times their weight.

53. The method according to claim 38, wherein the active ingredient is suspended in an aqueous medium.

54. The method according to claim 40, wherein the less poorly soluble active ingredient has the same kind of activity .

55. The method according to claim 41, wherein the collagen suspension is lyophilized to sponges.--

DESCRIPTION

ACTIVE INGREDIENT MATRIX IN THE FORM OF A BIOLOGICALLY RESORBABLE, POROUS NONWOVEN, METHOD FOR ITS MANUFACTURE AND USE THEREOF

[001] The invention relates to an active ingredient matrix in the form of a biologically resorbable, porous nonwoven of collagen fibrils in lyophilized form, a method for the manufacture of the active ingredient matrix and the use thereof.

[002] Over the past few years the need for resorbable hemostatics has led to the development of collagen-based products. Collagen sponges have been clinically used in large numbers for many years. As examples of surgical use reference is made to:

- capillary hemorrhages,
- parenchymatous hemorrhages,
- support measures for other hemostatic methods.

[003] Collagen sponges combined with commercially obtainable fibrin adhesion systems are used for arresting diffuse hemorrhages, particularly in parenchymatous organs.

[004] For some years products have been commercially available, which comprise aminoglycoside-filled collagen. These products are lyophilized solutions of dissolved collagen and gentamycin sulphate. The disadvantage of such lyophilized, antibiotic-containing collagen sponges is that the action of the active ingredient rapidly decreases following implantation.

[005] Patent DE 32 12 412 C2 describes a tissue-adherable, collagen wound overlayer. Isolated bovine tendons are homogenized and then the soluble collagen is extracted in citric acid solution or acetic acid pepsin solution. The thus extracted, dissolved collagen is mixed, after dialysis, with corresponding active ingredients such as antifibrinolytics and/or water-soluble antibiotics and then lyophilized.

[006] DE 31 24 981 A1 describes an active ingredient-containing collagen insert for insertion in bones or soft parts. Here the collagen is obtained as a citric acid extract from bovine tendons and this extract is provided with the active ingredient. The extract, comprising dissolved collagen and dissolved antibiotic is lyophilized. If said collagen insert is e.g. introduced into the tibia, it is completely resorbed there within three weeks and during this time antibiotic is released.

[007] European patent EP 360 180 B1 describes a method for the manufacture

of collagen foams in the form of continuous strips. Here again the collagen is dissolved in that a clearly defined part of the intermolecular bonds is cleaved. Cleaving takes place in such a way that most of the collagen is made water-soluble. The intramolecular bonds of the collagen are not attacked. However, the cleaving can also be controlled in such a way that only a small proportion of intermolecular bonds is cleaved, so that preponderantly higher molecular weight, water-insoluble collagen aggregates are obtained, which are dispersed in water. The aids used for bringing about controlled intermolecular cleaving are not described.

[008] Further methods for the manufacture of collagen sponges by lyophilization of collagen solutions or dispersions have long been known (e.g. EP 209 726, Beutler/Eblinger/Lindner; DE-OS 32 03 957, Eckmayer; US patent 3 157 524, Artandi; DE-OS 33 15 678, Cioca and DE 18 11 290, Chvapil).

[009] There is a need for creating an active ingredient matrix in which the active ingredient action persists over a longer period. In addition, the active ingredient matrix must be easy to manufacture, biologically compatible and able to absorb active ingredients or active ingredient combinations of different types.

[010] The object of the invention is an active ingredient matrix in the form of a biologically resorbable, porous nonwoven of collagen fibrils in lyophilized form with a long lasting release of active ingredients, containing at least one, homogeneously distributed active ingredient, which is difficultly soluble in water and body fluids.

[011] The active ingredient matrix according to the invention and which is suitable for implantation purposes as an active ingredient-containing collagen, fulfils the following requirements:

[012] The active ingredient or carrier matrix is sterilizable, resorbable, has no permanent tissue reaction, does not disturb wound healing, is easy to apply and offers large-area tissue contact.

[013] Active ingredient release is protracted and complete. It is possible to attain high local tissue levels, linked with a low systemic concentration.

[014] The invention also provides a method permitting the incorporation of active ingredient forms difficultly soluble in water, particularly medicaments, in a collagen suspension. Incorporation takes place in such a way that the homogeneity of active ingredient distribution is ensured.

[015] The object of the invention is a method for the manufacture of a biodegradable active ingredient matrix in the form of an open-cell nonwoven

of uncrosslinked, resorbable collagen fibrils, particularly of a bovine origin, which is characterized in that cleaned, degreased and dried hide portions are allowed to swell in dilute, organic acids until an elastic material is obtained, the swollen pieces are rinsed several times with aqueous media accompanied by an increase in the pH-value, the swollen pieces are mechanically separated into fibres for forming a suspension of collagen fibrils, the pourable collagen suspension having a pH-value of > 3.5 to < 4.8 is mixed with at least one difficultly soluble active ingredient in finely divided form and homogenized and the active ingredient-containing suspension is then lyophilized to the nonwoven.

[016] Preferably cattle hide is used as the starting material for the active ingredient matrix. For obtaining swellable pieces the hide, preferably after liming and removing the epidermis and subcutis, is comminuted to cube-like pieces, which can then be cleaned, degreased and dried, so that they are in a form suitable for swelling.

[017] The active ingredient matrix according to the invention advantageously has a layer thickness of 0.5 to 15 mm, particularly 2 to 5 mm. In the lower, thin area reference can be made to a nonwoven. In the upper, thick area the active ingredient matrix structure is similar to a sponge and can be referred to as such. The density of the nonwoven is generally between 12 and 180 mg/cm^3, particularly between 40 and 80 mg/cm^3. The higher the density the longer the resorption time, which also influences the active ingredient delivery time.

[018] Due to manufacture by lyophilization the pore volume of the active ingredient matrix according to the invention can be kept very high and is generally 60 to 80% of the total volume. The average pore size is roughly in the range 20 to 150 µm. The specific surface, measured according to Brunauer, Emmett and Teller (BET) is generally 150 to 350 m^2/m^2 of collagen nonwoven. This also leads to a high, preferred air permeability of 2,500 to 5,000 $ml/cm^2/min$, particularly 2,700 to 3,400 $ml/cm^2/min$, for a layer thickness of 4.2 mm.

[019] Body fluid-difficultly soluble active ingredients are those having in the physiological medium a solubility lower than 10 mg/ml and particular importance and advantage is attached to those active ingredients having a solubility equal to or lower than 1 mg/ml. Active ingredients can have a medicament function, as is generally the case. However, other substances can be used as active ingredients, such as e.g. diagnostics, where it can be just as important that they are slowly released over a longer time period.

[020] The following medicaments are suitable:

from the class of steroid antibiotics: fusidic acid;
from the class of sulphonamides: silver sulphadiazine;
macrolide antibiotics: erythromycin, rifampicin;
from the class of aminoglycoside antibiotics: clindamicin-palmitate clindamicin-palmitate hydrochloride (Sobelin®, Pharmacia and Upjohn) and gentamicin-crobefat (E. Merck, Darmstadt, EMD 46/217, EP 173 186 A1, example 9);
from the class of glycopeptides: vancomycin
from the class of quinolones: nalidixic acid, ciprofloxacin.

[021] The active ingredient content of the active ingredient matrix can vary within wide limits. It is generally between 3 and 30 wt.%, based on the total weight of the lyophilized active ingredient matrix and is in particular between 5 and 20 wt.%. The weight per unit area of the active ingredient matrix can vary within wide limits as a function of the layer thickness. It is generally between 1 and 50 mg/cm^2, particularly between 10 and 20 mg/cm^2.

[022] Among medicaments with antibiotic characteristics particular preference is given to the aminoglycoside antibiotics clindamicin-palmitate and clindamicin-palmitate hydrochloride, as well as gentamicin-crobefat. The active ingredient matrix according to the invention can also contain several difficultly soluble active ingredients, particularly with different action directions. In a similar manner it is also possible, besides the at least one difficulty soluble active ingredient, to provide one or more active ingredients with less difficult solubility or easy solubility in the active ingredient matrix. Here again it is possible to provide active ingredients with different action directions. Preference is generally given to the provision of active ingredients with the same action direction, but varying rapidity of release. Thus, e.g. in the case of antibiotics, it is possible to combine an antibiotic with retarded release such as gentamicin-crobefat with an antibiotic having the same action direction but a rapid release such as gentamicin-sulphate. This makes it possible to obtain desired, initial high tissue levels with respect to the antibiotic, associated with a long lasting, retarded release of the difficultly soluble antibiotic. The active ingredient matrix according to the invention is consequently eminently suitable for use as an implantable and resorbable depot for active ingredients with a retarded active ingredient delivery, optionally associated with active ingredients having a rapid active ingredient delivery.

[023] During the manufacture of the active ingredient matrix according to the invention it has been found that the handling of the suspension of collagen fibrils, which are largely present in isolated form in the suspension, can be significantly simplified if the suspension has a pH-value of > 3.5 to < 4.8. In this pH-range, the collagen suspension is pourable and makes it possible, following the addition of the active ingredients, to

homogeneously distribute the same in the suspension, so that they are present in the finished matrix in homogeneous form, embedded between the collagen fibrils. It is fundamentally possible to set the suitable pH-value following the mechanical separation into fibres by adding acid or base. However, it is advantageous for the method and product, to use a preferred procedure in which the acid concentration is so set during the swelling process that following the rinsing of the swollen collagen portions and during the subsequent separation into fibres of the portions into collagen fibrils a suspension is obtained where said pH-range automatically occurs. A pH-range between 4.0 and 4.5 is preferred. As the pH-range is adjustable by choosing the concentration of the organic acid combined with the number of rinsing operations and optionally the rinsing liquid quantity, there are sufficient variation possibilities for obtaining the desired result. Normally at least two rinsing operations and in particular at least five such operations are performed. In a rinsing cycle the rinsing water is removed after each rinsing operation. Generally rinsing or washing takes place with demineralized water. If the active ingredient is added in aqueous medium, e.g. in a suspension, then the latter preferably has roughly the same pH-value in order to avoid pH-shifts as a result of active ingredient addition. Due to the fact that demineralized water is used during rinsing or washing and there is no need for pH-correction after rinsing, the introduction of salts into the active ingredient matrix is avoided, so that the latter is substantially salt-free, which is desirable in many cases.

[024] Swelling can be carried out for a period of 5 to 60 hours and in particular 6 to 48 hours. The swelling period is dependent on the acid concentration and the origin of the collagen material. The organic acid concentration during the swelling process is normally 0.01 to 2 N, particularly 0.05 to 0.5 N and in general is 0.1 N. A suitable organic acid is acetic acid. Other organic acids can be used and preference is given to those having a biological compatibility. Volatile acids are particularly preferred, because they are removed during lyophilization.

[025] As a result of swelling the hide portions which, as stated hereinbefore, are in particular of a bovine origin, are swollen to 3 to 10 times, particularly 4 to 8 times their weight. The size of the precleaned, dry hide portions is advantageously chosen in such a way that after swelling pieces with a diameter of approximately 1 cm are obtained. This size is advantageous for handling and subsequent separation into fibres. Following on to the swelling operation and after removing the wash water, the swollen collagen granulate is received in demineralized water in order to prepare it for the subsequent separation into fibres. The water quantity is preferably chosen in such a way that a 0.1 to 10% mixture, based on the dry collagen material, is obtained. Separation into fibres preferably takes place by dispersion, accompanied by stirring and the collagen structure is broken up

whilst producing isolated collagen fibrils. After adding the at least one difficultly soluble active ingredient and optionally further, less difficultly soluble active ingredients to the suspension and further homogenization, the suspension is ready for lyophilization, without any other intermediate treatment being necessary.

[026] The invention is further illustrated hereinafter by the description of preferred embodiments, which also reveal the more detailed contexts, together with a performance example.

Swelling stage

[027] The cleaned cattle hide collagen is now swollen in a first working up stage with dilute acid, preferably 0.1 N acetic acid, over a period of 6 to 48 hours, preferably 16 hours. As a result of acid swelling from the hard, brittle cattle hide collagen is obtained an elastic material from which in a subsequent stage with the aid of a mechanical process (cutting process) the collagen fibrils can be isolated.

[028] The acetic acid concentration during the swelling stage is chosen at 0.1 N in such a way that the collagen suspension to be produced in the second stage has a pH-value of 4.0 to 4.5. If the pH-value of the collagen suspension to be prepared in the second stage is above 4.8, the collagen fibres are precipitated from the suspension and there is no longer a homogeneous solution. If the pH-value is below 3.5, the collagen mass is viscous and can no longer be processed.

Rinsing stage

[029] Using two test mixtures the influence of the rinsing cycles on the pH-value or acetic acid concentration in the rinsing water is tested.

[030] The pH-value is determined potentiometrically. The acetic acid concentration in the rinsing water is determined enzymatically.

[031] The following measured values are obtained:

Table 1: pH-value and acetic acid concentration for the rinsing solutions

Solution	pH-value test 1	pH-value test 2	Conc. acetic acid (mg/l) test 1	Conc. acetic acid (mg/l) test 2
0.1 N acetic acid mixture	2.85	2.82	6.37	6.40
16 h swelling with collagen	3.78	3.69	5.35	5.47
1st rinsing	3.90	3.94	0.36	0.33
2nd rinsing	3.85	3.85	0.16	0.12
3rd rinsing	3.88	3.95	0.12	0.07
4th rinsing	3.93	4.00	0.13	0.05
5th rinsing	3.98	4.05	0.08	0.05

[032] As can be gathered from the table a 5 times rinsing is sufficient for giving a pH-value of approximately 4.0 in the rinsing solution.

[033] As has already been stated, the acetic acid concentration of 0.1 N in the swelling process was chosen so that during the preparation of the collagen suspension a pH-value of 4.0 to 4.5 occurs. During the swelling process the collagen granulate weight has risen by a factor of 4 to 8 by a corresponding water absorption. It is now in the form of a soft, flexible, elastic material of dimensions 1 x 1 x 1 cm.

Mechanical comminution

[034] To the swollen collagen granulate is added sufficient water to provide a 0.1 to 10% suspension, which is homogenized in 2 to 15 minutes in a high power dispersing machine at 400 to 1200 r.p.m. As a result of the mechanical working up process a suspended, wide-mesh fibre braid is obtained, which no longer has any points in common with the in vivo arrangement of collagen fibres in the corium. The in vivo strong collagen fibres braiding through in all directions are broken up and are obtained in the form of shorter fragments.

[035] It has been found that a change in the pH-value of the collagen suspension by adding 0.1 N caustic soda solution or 0.1 N hydrochloric acid

leads to the following modifications in the consistency of the suspension:

Set pH-value of collagen suspension	Collagen suspension consistency
3.07	Gel, semifluid
3.60	Gel, semifluid
4.03	Pourable suspension
4.30	Pourable suspension *
4.52	Pourable suspension
5.02	Very highly fluid, fibril aggregation
5.55	Long collagen fibrils, separation of fibres and liquids

* Suspension prepared without acid and solution addition.

[036] The following observations were made at the different pH-values set on the collagen suspension:

[037] Below a pH-value of 3.6 the collagen suspension is semifluid. However, there is no agglomeration of fibres to fibre bundles. There is no separation of fibres and liquid.

[038] If to a collagen suspension of pH-value 3.6 is added the corresponding caustic soda solution quantity in order to obtain a pH-value of 4.3, then from the viscous gel once again a highly fluid suspension is obtained. On further increasing the pH-value (5.0), there is a separation of fibres and liquid and the individual fibres agglomerate to fibre bundles.

[039] The tests proved that in the preferred pH-range of 3.8 to 4.5 there is always a pourable collagen suspension, which can be further processed without difficulty.

Homogenization

[040] To the collagen suspension is now added the corresponding quantity of the difficultly soluble medicament.

[041] The term difficultly soluble medicaments here in particular covers active ingredients having in the physiological medium a solubility of < 1 mg/ml.

[042] After adding the difficultly soluble medicament the active ingredient-containing suspension is homogenized for 5 to 30 minutes accompanied by

stirring with an electric stirrer at 20 to 300 r.p.m. The active ingredients are introduced in the form of an aqueous slurry or in power form.

[043] Lyophilization of pharmaceutical products has been carried out for many years with the main aim of in particular bringing unstable medicaments into dry, storable forms from which it is possible to produce sterile medicaments.

[044] The most important stage in lyophilization is the freezing phase. In this phase the crystal lattice is created from which the following sublimation takes place. As the collagen suspension is not a homogeneous solution, but instead a multisubstance system with solid ingredients, of the conventionally performed tests for determining the freezing parameters such as the determination of the eutectic point, the determination of the collapse temperature and DSC measurements in the low temperature range, only the eutectic temperature was determined. Tests on several collagen suspensions from several collagen granulate batches gave a freezing point reduction to a range -2 to -4°C for a collagen suspension with 2 wt.% collagen. The eutectic temperature for the combination of collagen suspension and difficultly soluble medicament is preferably separately determined for each preparation. The data obtained during the determination of the eutectic point form the basis for the control of lyophilization. The prerequisite for a correct lyophilization for the active ingredient-containing collagen suspensions is that there is a clear drop below the eutectic temperature.

[045] Changes to the pH-value of the collagen suspension by adding caustic soda solution or hydrochloric acid leads to the following changes in the physical stability of the lyophilized nonwoven or the time necessary for complete wettability.

Table 3: Influence of pH-value of collagen suspension on the physical stability of the lyophilized collagen matrix and the time for complete wetting after immersion in water.

Set suspension pH-value	Complete wettability time (s)	Physical stability of lyophilized matrix
2.98	30.2	good
3.40	42.7	good
3.87	27.6	good
4.30	10.8	good
4.45	12.6	good
4.90	7.0	bad *
5.34	6.1	bad *

* Bad stability is understood to mean that the lyophilized fleece breaks down into individual fibre aggregates after placing in water.

[046] The tests show that collagen nonwovens with a good "wet" stability are always obtained from collagen suspensions in the pH-range 3.8 to 4.5.

[047] The following further conclusions are made:

- pH-values < 3.8 give a slower wettability than nonwovens with pH-values > 3.8;
- for set pH-values 4.5, there is a pronounced decrease in the stability of the nonwovens produced;
- the optimum pH-value setting for the collagen suspension is between 3.8 and 4.5;
- if the collagen suspension pH-value is above 4.8, the collagen fibres are precipitated from the collagen suspension and there is no longer a homogeneous solution.

[048] The active ingredient-containing collagen matrix obtained from cattle hide can give as a result of multistage mechanical and chemical working up processes a wide-mesh fibre braid (light microscopic result), which no longer has any points in common with the in vivo arrangement of collagen fibres in the corium. The in vivo-strong collagen fibre bundles braiding through in all directions are broken up and are obtained as shorter fragments, so that a system is obtained with wide interfibrillar gaps. During lyophilization a coarse meshed network is obtained which, considered spatially, can be compared with a natural sponge. A very large, inner surface increase is obtained, which is formed by collagen fibres.

[049] Electron microscopic section preparations reveal a pattern of collagen fibrils in the form of varyingly thick bundles, which run in all directions in space. Thus, there are longitudinal, transverse and tangential sections of collagen fibrils. Longitudinal fibrils have a typical transverse

striping. The surface relief of the lyophilized collagen suspensions rendered visible by scanning electronic microscopy is particularly impressive. Thinner fibres branch off to either side of strong fibre bundles. Where several thin fibres meet, frequently knot-like thickenings arise. In addition, there are strong collagen fibrils twisted in rope-like manner.

[050] In each case 1 cm^2 pieces are cut from two different collagen implants (matrix with clindamicin-palmitate and matrix with clindamicin-palmitate hydrochloride). The pieces are weighed and the weight recorded. Subsequently the active ingredient-containing implants are placed in test tubes with in each case 5 ml of 0.066 M phosphate buffer (pH = 7.4) or human serum and eluted in the water bath at 37°C. After 24 hours the implant is removed and placed in fresh buffer solution or fresh serum. The total elution time is 10 days for a 24 hour buffer change. The biological measure used for the antibiotic concentration is the inhibiting action (determining the inhibition halo diameter) on the test organism Staphylococcus aureus ATCC 6538 and Micrococcus luteus DSM 348. The inhibiting action of the sample is compared with the inhibiting action of stepped doses of a standard (here clindamicin hydrochloride). From identically large inhibition halos for samples and standards, it can be concluded that there are identical antibiotic concentrations.

[051] The delivery of the antibiotics from the different implants can be gathered from table 4.

Table 4: Active ingredient delivery of clindamicin-palmitate as a function of the elution time

Days	Clindamicin-palmitate in phosphate buffer (µg implant)	Clindamicin-palmitate hydrochloride in phosphate buffer (µg implant)	Clindamicin-palmitate in serum (µg implant)
1	18.3	16.0	8.0
2	46.1	23.8	21.2
3	46.1	46.1	34.7
4	52.7	35.4	29.1
5	46.1	46.1	36.1
6	60.1	46.1	37.8
7	46.1	35,4	22.1
8	18.3	20.8	16.5
9	12.3	7.2	10.8
10	12.3	5.5	8.1

[052] The table clearly reveals that the antibiotic delivery from both implants is still not ended after 10 days. The antibiotic quantity to be delivered is approximately the same per unit of time for clindamicin-palmitate ester and free palmitate ester.

[053] To check whether antibiotic residues have been left on the carrier material, the implants were placed after a 10 day elution period on an agar surface contaminated with Staphylococcus aureus or Micrococcus luteus and incubated at 37°C. After this time period antibiotic could still be detected in the collagen sponges.

[054] If there is a demand for implants able to protect a wound area over a 5 to 15 day period, the materials described here are suitable for ensuring this protection. There is a combination of matrix and active ingredient, which releases the active ingredient in delayed form and in suitable concentrations over a 5 to 15 day period. In addition, up to complete resorption the matrix is protected against bacterial colonization by the adhering biotic. In the investigation of the active ingredients clindamicin-palmitate and clindamicin-palmitate hydrochloride it was surprisingly found that contrary to the results published in the literature (Antimicrobial Chemotherapy, published by David Green Wood, 3rd edition, Oxford University Press, 1995), clindamicin esterified with palmitic acid also reveals an antibiotic activity. In the case of elution in the aqueous buffer system, surprisingly antibiotic activity was detected over a period of at least 11 days. The esterification of the OH-group of clindamicin with palmitic acid admittedly drastically reduces clindamicin solubility (see table 5), but does

not lead to an inactivation of the molecule with respect to its antibiotic activity. This means that the clindamicin-palmitate does not first have to be cleaved by esterases into the active molecule clindamicin. This is important, because as a result the use of a clindamicin-palmitate-containing collagen matrix is not dependent on the presence of enzymes.

Table 5: Irregular solubility behaviour of the palmitate ester of lincomicin and clindamicin*

pH	Lincomicin-palmitate hydrochloride (mg/ml)	pH	Clindamicin-palmitate hydrochloride (mg/ml)
2.3	0.124	3.7	53.2
-	-	5.8	< 0.001
7.7	0.0249	7.4	< 0.002

Literature: E. L. Rowe, Journal of Pharmaceutical Sciences, vol. 68, no. 10 (1979), pp 1292-1296.

Performance example

1. Swelling stage

[055] After swelling, 1823.1 g of collagen granulate (water content 15.0%, average granulate size 4 to 6 mm) are rinsed in 36 l of 0.1 N acetic acid for 16 hours 5 times with 5 l of water for injection purposes. After the fifth rinsing the pH-value of the rinsing solution must be > 4.0, otherwise further rinsing is needed.

2. Mechanical comminution

[056] Then topping up with water for injection purposes takes place to a total weight of 90.0 kg, whilst taking account of the subsequently to be added quantity of difficultly soluble medicament. The above suspension is homogenized for 2 minutes at 680 r.p.m. in a high power dispersing machine. The pH-value of the homogenized mass is checked.

3. Homogenization

[057] To the collagen suspension are now added 224.48 g of clindamicin-palmitate hydrochloride (corresponding to 136.26 g of clindamicin base) suspended in 5 l of 0.1 N acetate buffer at pH 3.6 to 3.8. The active ingredient-containing suspension is again homogenized for 15 minutes with an electric stirrer at 150 r.p.m. The total mass of the active ingredient-containing suspension is now 90.0 kg.

4. Lyophilization

[058] For determining the pouring weight on the lyophilization dishes (size 45.6 x 45.6 cm), the necessary active ingredient-containing collagen suspension quantity per nonwoven is related to the available surface area per lyophilization dish.

[059] The plat size of the collagen nonwovens after lyophilization is 43.5 cm x 43.5 cm = 1892.15 cm^2. An active ingredient-containing collagen nonwoven of dimensions 5 x 8 cm = 40.0 cm^2 contains 35 mg of clindamicin and 400 mg of anhydrous collagen.

$$\text{Clindamicin-base/dish} = \frac{1892.25\ cm^2}{40\ cm^2} \times 0.035\ g = 1.6557\ g$$

Thus: $\frac{1.6557\ g}{136.26\ g} \times 90000.0\ g = 1093.6\ g$ collagen-suspension are to be poured

on each lyophilization dish.

[060] The filled lyophilization dishes are introduced into the lyophilization plant. The following details apply to the lyophilization process:

charging the plant:	+ 7°C
product temperature on freezing:	+ 7°C -45°C
chamber internal pressure on freezing:	10^3 mbar
product temperature during main drying:	- 45°C +38°C
chamber internal pressure during main drying:	0.9 mbar
product temperature during redrying:	+ 38°C +21°C
chamber internal pressure on redrying:	0.03 mbar

5. Sterilization

[061] Sterilization takes place by radiation sterilization with 25 kGy or ethylene oxide.

CLAIMS

1. Active ingredient matrix in the form of a biologically resorbable, porous nonwoven of collagen fibrils in lyophilized form with a retarded release of active ingredients, containing at least one homogeneously distributed active ingredient difficultly soluble in water and body fluids, which, apart from the collagen fibrils as the carrier structure and the at least one active ingredient, is substantially free from further constituents, which is substantially free from salt and in the at least one active ingredient in physiological medium has a solubility of < 10 mg/ml.

2. Active ingredient matrix according to claim 1, characterized in that it has a layer thickness of 0.5 to 15 mm, particularly 2 to 5 mm.

3. Active ingredient matrix according to claim 1 or 2, characterized in that it has a density of 12 to 180 mg/cm^3.

4. Active ingredient matrix according to one of the claims 1 to 3, characterized in that it has a pore volume of 60 to 80% of the total volume.

5. Active ingredient matrix according to one of the preceding claims, characterized in that it has an average pore size in the range 20 to 150 µm.

6. Active ingredient matrix according to one of the preceding claims, characterized in that it has an air permeability of 2500 to 5000 ml/cm^2/min, particularly 2700 to 3400 ml/cm^2/min, for a layer thickness of 4.2 mm.

7. Active ingredient matrix according to one of the preceding claims, characterized in that the at least one difficultly soluble active ingredient is a medicament, particularly an antibiotic.

8. Active ingredient matrix according to claim 7, characterized in that as the antibiotic are used aminoglycoside antibiotics, particularly clindamicin-palmitate, clindamicin-palmitate hydrochloride and/or gentamicin-crobefat.

9. Active ingredient matrix according to one of the preceding claims, characterized in that in addition to the at least one difficultly soluble active ingredient, it contains at least one less difficultly soluble or easily soluble active ingredient.

10. Method for the manufacture of biodegradable active ingredient matrix in the form of an open-cell nonwoven or sponge of uncrosslinked, resorbable

collagen fibrils, particularly for the manufacture of an active ingredient matrix according to one of the preceding claims, characterized in that pieces of cleaned, degreased and dried hide are allowed to swell in dilute, aqueous solutions of organic acids until an elastic material is obtained, the swollen pieces are rinsed several times with aqueous media, particularly demineralized water until the pH-value is increased, the rinsed pieces are mechanically separated into fibres for forming a suspension of collagen fibrils, the pourable collagen suspension having a pH-value of > 3.5 to < 4.8 is mixed with at least one difficultly soluble active ingredient in finely divided form and homogenized and the active ingredient-containing suspension is then lyophilized to the nonwoven or sponge.

11. Method according to claim 10, characterized in that the concentration of the organic acid used for swelling and the number of rinsing operations are chosen and matched to one another in such a way that following the rinsing and separation into fibres, without prior pH-correction, a collagen suspension is obtained with a pH-value of > 3.5 to < 4.8, particularly 4 to 4.5.

12. Method according to claim 10 or 11, characterized in that the rinsing operation covers at least two, particularly at least five rinsing cycles.

13. Method according to claim 11 to 12, characterized in that rinsing is performed for 5 to 60 hours, particularly 6 to 48 hours.

14. Method according to claim 11 to 13, characterized in that for swelling purposes use is made of an acid solution with an acid concentration of 0.01 to 2 N, particularly 0.05 to 0.5 N.

15. Method according to claim 11 to 14, characterized in that the hide portions are swollen in the organic acid to 3 to 10 times, particularly 4 to 8 times their weight.

16. Method according to claim 11 to 15, characterized in that, after rinsing and removing the rinsing water, the swollen collagen granulate is transformed by the addition of water into a 0.1 to 10% mixture, based on the dry collagen material weight and this mixture is homogenized by dispersion to the collagen suspension, the fibre union of the collagen fibrils being broken.

17. Method according to claim 11 to 16, characterized in that the at least one difficultly soluble active ingredient is added in finely divided form, particularly suspended in an aqueous medium.

Amended sheet

18. Method according to one of the claims 11 to 17, characterized in that the suspension of the collagen fibrils, following the addition of the at least one difficultly soluble active ingredient, is homogenized for uniform distribution of the at least one active ingredient in the suspension.

19. Method according to claim 11 to 18, characterized in that apart from the at least one difficultly soluble active ingredient, at least one less difficultly soluble active ingredient, preferably with the same action direction is added.

20. Method according to claim 11 to 19, characterized in that the homogenized, active ingredient-containing collagen suspension is lyophilized without any further intermediate treatment to in particular areal nonwovens or sponges.

21. Use of the active ingredient matrix according to one of the claims 1 to 9 as an implantable and completely resorbable depot for active ingredients with a retarded active ingredient delivery.

DECLARATION FOR PATENT APPLICATION	Attorney Docket 24669

As a below-named inventor(s), I/we hereby declare that

My/Our residence(s), post office address(es) and citizenship(s) is/are as stated below next to my/our name(s)

I/We believe I/we am/are the original inventor, first and sole (*if only one name is listed below*) or the original, first and joint inventors (*if plural names are listed below*) of the subject matter which is claimed, and for which a patent is sought on the invention entitled **active ingredient matrix in the form of a biologially resorbable porous nonwoven, method for its manufacture and use** the specification of which (check one) **thereof**

[ ] is attached hereto

[X] was filed on June 7, 2001, as Serial No. 09/857,554,

and was amended on _____ 19____ (if applicable)

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of this application as defined by 37 CFR § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Applications:

			Priority Claimed	
198 56 668.9 (Application No.)	Germany (Country)	09 12 /1998 (Day/Month/Year Filed)	[X] Yes	[ ] No
_____ (Application No.)	_____ (Country)	/ / (Day/Month/Year Filed)	[ ] Yes	[ ] No
_____ (Application No.)	_____ (Country)	/ / (Day/Month/Year Filed)	[ ] Yes	[ ] No

We hereby appoint Gary M. Nath, Reg. No. 26,965; Harold L. Novick, Reg. No. 26,011; Suet M. Chong, Reg. No. 38,104; Todd L. Juneau, Reg. No. 40,669; Patricia M. Drost, Reg. No. 29,790; Lee C. Heiman, Reg. No. 41,827; Jerald L. Meyer, Reg. No. 41,194; Joshua B. Goldberg, Reg. No. 44,126; David Milligan, Reg. No. 42,893 and Robert G. Lev, Reg. No. 30,280; David R. Murphy, Reg. No. 22,751; Paul A. Sacher, Reg. No. 43,418; Gregory B. Kang, Reg. No. P-45,273; Scott F. Yarnell, P-45,245; as my attorneys to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith.

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We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by 35 U.S.C. § 112, first paragraph, I/we acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

_____ (U.S. Application Serial No.)	_____ (U.S Filing Date)	_____ (Status--patented, pending, abandoned)
_____ (U.S. Application Serial No.)	_____ (U.S Filing Date)	_____ (Status--patented, pending, abandoned)

DECLARATION FOR PATENT APPLICATION	Attorney Docket: 24669

We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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Country of Citizenship: _____

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